ABSTRACT

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Title of diploma thesis:
Optimalisation of methods for determination of CD44 receptor activation.

CD44 is a transmembrane glycoprotein present in most cell types which is involved in many physiological and pathological processes and it represents the main hyaluronic acid (HA) receptor. HA is a nonsulfated glycosaminoglycan that is present extensively in extracellular matrix, where it provides cell hydratation. HA is involved in cancer, affects cell migration, supports an angiogenesis and participates in wound healing. In this study, we focused on the optimization of methods for the evaluation of activation, the amount and cleavage of CD44 receptor in HT-29 cell line.

We observed the total amount of the receptor and its cleaved domains using methods Western Blot, ELISA and flow cytometry. We used HA molecular weight 71 kDa and PMA (Phorbol-12-Myristate-13-Acetate) for activation. Time-dependent changes appeared in the amount of total CD44 and its fragments after activation. We also observed cell death depending on the presence of HA and PMA. We demonstrated no significant effects of these substances on cell viability. We used fluorescent and confocal microscopy for visual evaluation of the cleaved intracellular domain localization. In the presence of PMA, we observed the GFP-tagged intracellular domain translocation inside the nucleus in time.

In this work, we have managed to help optimize methods, which can be used to observe the CD44 receptor activation in HT-29 cell line.

Keywords: hyaluronic acid, CD44, Western Blot, ELISA, flow cytometry, microscopy